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Isolation and Characterization of Two cDNA Clones Coding for Isoforms of the *Parietaria judaica* Major Allergen Par j 1.0101

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Abstract

Two cDNA clones named P9* and P1* of 794 and 631 bp, respectively, were isolated from a λ ZAP cDNA expression library using *Parietaria judaica* (Pj) pollen-specific IgE antibodies from a pool of sera ($n = 23$) of patients allergic to Pj. Sequence analysis showed open reading frames of 176 and 138 amino acids. Both clones contain a putative signal peptide giving two mature processed proteins named Par j 1.0102 of 14,726 D and Par j 1.0201 of 10,677 D. These proteins represent isoallergenic forms of the major Pj allergen Par j 1.0101 (clone P5) previously reported. The Par j 1.0102 shared 98% amino acid sequence homology with the P5, while the Par j 1.0201 shared 89% homology. Since P1, P5 and P9 clones were expressed in *Escherichia coli*, and since the three allergenic proteins shared a very high degree of sequence identity and comparable binding to the Pj-specific IgE, we decided to analyze in more detail the immunological properties of only one allergen, the recombinant Par j 1.0101. The allergenic activity determined by the histamine release assay ranged between 9 and 56%, depending on the allergic patient analyzed, while it blocked approximately 40% of all the Pj-specific IgE antibodies, as detected after ELISA and cross-absorption analysis.

Key Words

Parietaria judaica
Recombinant allergen
IgE
Isoallergen

Introduction

The molecular characterization of allergens has received an important boost from recombinant DNA technology making possible to obtain purified soluble allergens or their subfragments from several allergenic sources [1–3]. The nucleotide and the deduced amino acid sequences of many allergens represent a powerful tool for the determination of the specific regions capable of interacting with IgEs and T cells, a task that was reached only with some difficulty a short time ago. These regions have recently received a great deal of attention since they could be useful in the development of reagents and novel therapies for the treatment of

allergies [4, 5]. In order to facilitate the amino acid determination of the B and T cell epitopes, the information derived from the amino acid sequence of isoforms or of highly related allergens could be quite important [6–10].

The pollen of the weed *Parietaria judaica* (Pj) is the main cause of allergic reactions in the Mediterranean area, and up to 50% of the allergic patients show specific IgE towards Pj pollen [11]. A Pj pollen crude extract contains at least nine different allergens with a molecular weight ranging from 14 to 80 kD [12, 13]. One of them, the Par j 1.0101 formerly *Par j1*, has been already cloned and sequenced [3]. It is a protein of 139 amino acids and a deduced molecular weight of 14,509 D, and it has been shown to be a major

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allergen since it reacts with 95% of the sera of Pj-allergic patients [3].

The aim of this study was to isolate and characterize isoallergenic forms of the Pj major allergen Par j 1.0101 in order to gain additional information on the IgE epitope localization, and in the present paper we describe the identification and characterization of two cDNA clones coding for isoforms of the Par j 1.0101.

Materials and Methods

Total RNA and Poly(A)⁺ RNA Preparation

The total RNA was extracted from the Pj flowers collected in Palermo, Italia, and the poly(A)⁺ RNA prepared as previously reported [3].

cDNA Library Screening and cDNA Sequencing

Double-strand cDNA was synthesized from mRNA using oligo dT primer and cloned into the *Eco*RI and *Xho*I sites of the ZAP expression vector, according to published procedures in the Stratagene cDNA cloning kit (Stratagene, La Jolla, Calif., USA). In brief, a ZAP cDNA expression library containing about 1.2×10^6 recombinant phages was constructed from poly(A)⁺ RNA of Pj inflorescence. 6×10^5 plaques were immunologically screened using pooled sera ($n = 23$) from patients allergic to Pj pollen, and 10 positive IgE-binding plaques were obtained. The nucleotide sequence of the cDNA insert was determined on both strands of PBK-CMV by the dideoxy sequencing procedure (Sequenase Amersham, Little Chalfont, UK).

Expression of Recombinant Protein

The whole cDNA insert of the clone P5 coding for the Par j 1.0101 was subcloned into an *Eco*RI site of the pMalC2 expression vector (BioLabs, Beverly, Mass., USA) and the clone named pMalP5 gave a maltose-binding protein (MBP) fused to the Par j 1.0101 with a deduced molecular weight of 58 kD. The whole cDNA inserts of clones P9 and P1 were subcloned into *Eco*RI-*Xho*I sites of the pGEX-10 expression vector and the clones named pGEXP9 and pGEXP1 gave a glutathione S-transferase protein fused to the Par j 1.0102 and Par j 1.0201 with a deduced molecular weight of 44 and 40 kD, respectively. The expression of the recombinant proteins was carried out according to the procedures outlined by the BioLab kit and Smith and Johnson [14].

Preparation of Soluble lysate and Purification of the Recombinant Allergen Par j 1.0101

The recombinant clone pMalP5 was grown to 0.5–0.6 OD A_{600} in LB broth and induced for 2 h with 0.3 mM of isopropylthio-B-D-galactoside. The cells were harvested by centrifugation (4,000 g for 20 min) and stored frozen. After thawing, the pellet was resuspended in a phosphate buffer (sodium phosphate 10 mM pH 7.2, 200 mM NaCl, 1 mM EDTA, 1 mM Na₂S₂O₃) and lysed by sonication using a Heat-System-Ultrasonic, W-385. The cell debris was removed by centrifugation (9,000 g for 30 min). The supernatant was diluted (1:20) with 10 mM EDTA and concentrated to 2 mg/ml of total proteins using a Centriprep concentrator (Amicon) with a molecular weight cutoff of 10 kD.

The Par j 1.0101 fusion protein was purified by acrylamide gel column electrophoresis [15], since the fused recombinant protein bound

very poorly to the amylose resin column. The concentration of the recombinant allergens was detected by densitometry analysis of PAGE-SDS gels stained with Coomassie brilliant blue.

Western Blot Analysis

The Western blot analysis was performed as previously reported [3].

Human Sera and Basophil Donors

The sera used ($n = 23$) were collected from subjects not previously hyposensitized, with a clear clinical history of seasonal allergy towards the pollen of Pj and with a high level (RAST class 4+) of IgE versus Pj allergens as determined by radioallergosorbent test (RAST). Control sera ($n = 5$) were obtained from subjects nonallergic to the Pj pollen and with negative values (0–5% of radiolabeled anti-human-IgE bound) after RAST analysis. The basophils for the histamine release assay were derived from subjects ($n = 6$) allergic to the Pj pollen having positive RAST values (classes 3+ and 4+).

The sera and the basophil cells were provided from volunteers after an informed consent.

Histamine Release (HR) Assay

The histamine released on challenge with the purified recombinant protein expressed by the pMalP5 clone was assayed by a single isotope enzymatic microassay as previously reported [16] with minor modifications [17]. In brief, we used 500 mCi/mmol S-adenosyl-L-methyl-(³H)-methionine (SAM; Amersham Radiochemical Center, UK) in conjunction with a crude preparation of rat kidney histamine-methyltransferase (HMT) [18]. To each triplicate aliquot of supernatant (20 μ l) obtained after release, 10 μ l of HMT-SAM solution were added and gently mixed. The tubes were incubated at 37°C for 1 h, and the reaction was terminated by addition of 10 μ l of 1 N NaOH.

The ³H-methyl-histamine formed was extracted with 250 μ l of chloroform [16]. The aqueous layer was collected after an additional washing step with 100 μ l of 1 N NaOH; 200 μ l of the chloroform-extracted material were transferred into a scintillator vial and evaporated to dryness. The bound radioactivity was measured after addition of the scintillator liquid. The total histamine contained in the basophils was determined from the supernatant obtained by boiling 200 μ l of blood for 10 min. The histamine released was expressed as percentage of the total histamine after subtraction of the level of histamine spontaneously released in the absence of the stimulus.

Rabbit Antibodies

Rabbit anti-Par j 1.0101 antibodies were raised by immunizing two rabbits with the purified recombinant protein expressed by the pMalP5 clone. The immunization and isolation of the rabbit immunoglobulins were performed as described by Harboe and Ingild [19]. The antibodies obtained were stored at 4°C in 15 mM sodium azide.

Immunoelectrophoretic Methods

Crossed Radio/immunoelectrophoresis (CRIE/CIE) were performed as described [12] by using the rabbit antibodies against the Par j 1.0101 and the serum pool of Pj-allergic subjects reported above.

Enzyme-Linked Immunosorbent Assay (ELISA)

The assay was essentially performed as an ELISA inhibition by using the total crude extract of the Pj pollen as substrate and the lysate of the recombinant pMalP5 clone as competitor of the specific IgE binding to the allergens present in the crude extract. Freshly prepared

Pj pollen crude extract was diluted to 0.1 µg/ml in coating buffer (0.01 M Na₂CO₃; 0.03 M NaHCO₃; 0.02% NaN₃, pH 9.6), and 0.2 ml were incubated overnight at room temperature in each well of polystyrene microtiter plates. The coated plates were washed three times with phosphate buffered saline, pH 7.2, containing 0.05% Tween 20 (PBS-Tween) and incubated for 6 h at room temperature with the serum pool diluted 1:20 (0% inhibition). The inhibition was performed by adding, to the diluted serum pool (1:20), increasing amounts of the lysate from the recombinant pMalP5 clone, for 3 h at room temperature. After three additional washing steps, the alkaline phosphatase conjugated to anti-human IgE was added, followed by incubation with *p*-nitrophenyl phosphate as substrate, under the conditions described [20]. The inhibition reference value (100% inhibition) was obtained by using the Pj pollen crude extract.

Cross-Absorption Analysis

Slot blot filters were prepared by using a slotmanifold [21] applied on nitrocellulose membrane (Amersham).

3 µg/slot of Pj crude extract and 5 µg/slot of lysate from either the pMalC2 vector as negative control or the recombinant pMalP5 clone were used. The filters were air-dried, treated for 30 min at room temperature in blocking solution (3% BSA, 0.5% Tween, 0.02 NaN₃ in 1×PBS) and then washed using 1×PBS with 0.1% Tween. The inhibition was performed by adding to the diluted serum pool increasing amounts of Pj crude extract, lysate of pMalC2 or the recombinant pMalP5, for 3 h at room temperature. The filters were then incubated overnight at room temperature with preabsorbed serum. After the washing steps, the filters were incubated for 1 h at room temperature with horseradish-peroxidase-conjugated rabbit immunoglobulins to human IgE (Sigma, St. Louis, Mo., USA). The final reaction was determined with the ECL detection system (Amersham). The intensity of the signal was measured using a Biorad densitometer (model GS-670).

Results

Isolation and Characterization of the Par j 1.0101

Isoforms

Ten IgE-binding clones were isolated after screening of a λ ZAP cDNA library, and seven clones were still positive after several purification steps.

The purified clones gave no IgE binding when tested with sera (n = 5) from subjects not allergic to the Pj pollen while they were capable of reacting with a monoclonal antibody specific for the Par j 1.0101 [22]. Sequence analysis showed only two independent clones, named P9 and P1. The nucleotide sequences of the P9 and P1 clones were compared (fig. 1A) with the P5 cDNA sequence already published [3]. The P9 clone showed an insert of 794 nucleotides with a deduced amino acid sequence of 176 amino acids and a molecular weight of 18,450 D. The N-terminal sequence of 37 amino acids, underlined in figure 2, is characteristic of the eukaryotic signal peptide sequence of glycosylated proteins. The putative mature protein should start from amino acid 38 giving a protein of 139 amino acids, with a mo-

lecular weight of 14,726 D. Since the P9 clone contains a 3' untranslated region identical to the equivalent region of the P5 clone, the P9 cDNA represents a full length isoform of the Par j 1.0101 coding for the unprocessed protein. In particular, the P9 and the P5 clone showed 99% homology at the nucleotide level (fig. 1A, B) and 96% identity at the amino acid level (fig. 2).

The sequence analysis of the P1 clone showed a 637 nucleotide insert (fig. 1A) with a deduced amino acid sequence of 139 amino acids and a molecular weight of 14,400 D. The N-terminal sequence of 36 amino acids, underlined in figure 2, showed properties of an eukaryotic signal peptide sequence of glycosylated proteins. The putative mature protein should start from the amino acid 37, giving a protein of 102 amino acids, with a molecular weight of 10,677 D. The coding region of the P1 clone showed 91% homology at the nucleotide level (fig. 1A, B) and 89% identity at the amino acid level with the P5 clone (fig. 2). No homology was detected in the 5' and 3' untranslated region. Therefore, the P1 clone represented a variant of the P5 clone, and it was designed as Par j 1.0201, according to the allergen nomenclature suggested [23]. The amino acid comparison between the P5, P9 and the P1 clone is reported in figure 2.

Allergenic and Antigenic Properties of the Recombinant Allergen Par j 1.0101

The recombinant protein Par j 1.0101 expressed by the pMalP5 clone showed allergenic activity since, in addition to the capacity of binding IgE from Pj-pollen-allergic subjects, as clearly demonstrated by the results reported in the Western blot analysis (fig. 3), it was also capable of inducing release of histamine from basophils of subjects (n = 6) allergic to the Pj pollen (table 1). The HR values ranged between 9 and 56%, depending on the basophils of the allergic subject analyzed. Basophils obtained from a nonallergic subject (D.G.) gave no detectable HR (table 1). The allergenic activity expressed by the purified pMALP5 protein (fig. 4) was relatively strong and approximately comparable with the activity expressed by the Pj total crude extract (table 1). The Par j 1.0101 was capable of inhibiting approximately 40% of all the IgE specific for the allergenic molecules present in a total crude Pj pollen extract (fig. 5). The inhibition analysis was carried out with a pool of sera (n = 23) from Pj-allergic patients and by two different methods, i.e. ELISA (fig. 5) and cross-absorption (data not shown) obtaining comparable results.

The substrate used in either ELISA and cross-absorption analyses was the Pj pollen crude extract. When the pMalC2 *Escherichia coli* lysate was used as a control, no inhibition was detected (fig. 5). The recombinant allergen Par j 1.0101

```

1
P9 5' caciaaagaacactgca
P1 5' aattccgaaaaatcaaaatcaaaagcaaaatctgcaagtacta-----a--

61
P9 ctcaccaacacaataatcctttcccaacctccaaacATGAGGACCGTCTCGGCTCCCTCG
P1 -----g-----***-----c-----G-----GT--

121
P9 GCGGTGGCGCTCGTGGTTATCGTGGCGGCAGGACTTGCATGGACCTCTTGGCTTCGGTG
P1 T-----TG-----TG-----C-----

181
P9 GCGCCTCCTGCCCCAGCCCCGGAAGCGAAGAACTGCGGGACTGTGGTGAGAGCGCTG
P5 5' caagaaacctgctgggactATG---A-----
P1 --A---***-----T-----T-----A---CG---G-----

241
P9 ATGCCGTGCCTGCCGTTTCGTGCAGGGGAAAGAGAAAGAGCCGTCAAAGGGGTGCTGCAGC
P5 -----C-----A-----C
P1 -----T-----C-----T-----

301
P9 GGCGCCAAAAGATTGGACGGGGAGACGAAGACGGGGCTGCAGAGGGTGCACGCTTGTGAG
P5 -----T-----C-----
P1 -----G-----C-----

361
P9 TGCATCCAGACCGCCATGAAGACTTATTCGACATCGACGGGAACTCGTCAGCGAGGTC
P5 -----T-----G---C-----
P1 -----C-----C---G-----

421
P9 CCCAAGCACTGCGGCATCGTTGACAGCAAGCTCCCGCCCATTGACGTCAACATGGACTGC
P5 --C-----G-----
P1 --A-----A-----

481
P9 AAGACACTTGAGTGGTTCCTCGGCAACCCCACTTCCAGTCTCTCTCCGTGATGGTCCC
P5 -----T-----GG---CT-GGC---CCCC-A-----
P1 -----C-----TC---AC-ACA---GGAA-Ttagggag-t-g-t-ttc---act-t--

541
P9 GTCACGGGCCCCAAGTGATCCCGCCACAAAGCACGGTTGGAGAGACCCAGATTAGAGTT
P5 -----
P1 aagtgtt-agagataa--aa-ttatggc-t-ttgaa-aatgatgtat-at-tgggtgaat

601
P9 CCGCCCCCGCACCGGAAAAAGCtaaccaagctttctccactcttgatgagcgaggct
P5 -----c-----
P1 -tattgttatacat----t--aa---tttgttg--at 3'

661
P9 aatattattaagccctaaactctaataacggtggaactgtgttatgagaagttatatgca
P5 -----

721
P9 ataattatcttaatatatatatatatatagttgactagtgagtagtgacatcattc
P5 -----

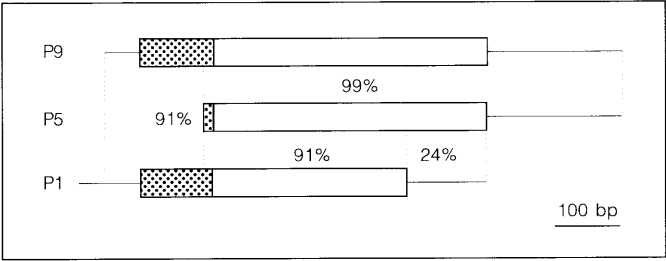
781
P9 acgtaataaataatgtgtactttacttactgatataatgattaaagatatgttgatt 3'
P5 ----- 3'

```

Fig. 1A

(For legend see p. 352)

Fig. 1. A Comparison of the nucleotide sequence of P1, P5 and P9 cDNA clones. The nucleotide sequence of the P9 clone is fully described. The nucleotide homology with the P1 and P5 cDNA is indicated with dashes, while asterisks indicate gaps. The sequence data for the P5 clone are from Costa et al. [3]. Capital letters indicate the nucleotide open reading frame. **B** Schematic drawing of nucleotide homology. The hatched boxes indicate the signal peptide, the empty boxes indicate the coding regions.



1B

	1	25
(P9) Par j 1.0102	<u>M R T V S A P S A V A L V V I V A A G L A W T S L</u>	
(P1) Par j 1.0201	- - - - - R - S - - - - - V - V - - - S	
	26	50
(P9) Par j 1.0102	<u>A S V A P P A P A P G S E E T C G T V V R A L M P</u>	
(P5) Par j 1.0101	- - - - - * - - - - - Q - - - - - M - R - - - -	
(P1) Par j 1.0201	- - - - - * - - - - - E - - - - - V - G - - - -	
	51	75
(P9) Par j 1.0102	<u>C L P F V Q G K E K E P S K G C C S G A K R L D G</u>	
(P5) Par j 1.0101	- W	
(P1) Par j 1.0201	- G	
	76	100
(P9) Par j 1.0102	<u>E T K T G L Q R V H A C E C I Q T A M K T Y S D I</u>	
(P5) Par j 1.0101	- - - - - P - - - - - - - - - - - - - - - D -	
(P1) Par j 1.0201	- - - - - P - - - - - - - - - - - - - - - Q -	
	101	125
(P9) Par j 1.0102	<u>D G K L V S E V P K H C G I V D S K L P P I D V N</u>	
(P5) Par j 1.0101	- -	
(P1) Par j 1.0201	- -	
	126	150
(P9) Par j 1.0102	<u>M D C K T L G V V P R Q P Q L P V S L R H G P V T</u>	
(P5) Par j 1.0101	- -	
(P1) Par j 1.0201	- - - - - - - - - - - L H Y K G N	
	151	175
(P9) Par j 1.0102	<u>G P S D P A H K A R L E R P Q I R V P P P A P E K</u>	
(P5) Par j 1.0101	- -	
	176	
(P9) Par j 1.0102	<u>A</u>	
(P5) Par j 1.0101	-	

Fig. 2. Comparison of the amino acid sequence of Par j 1.0101, Par j 1.0102 and Par j 1.0201. The amino acid sequence (one-letter code) of the Par j 1.0102 is fully reported. The homology with the other two sequences is indicated with dashes, while asterisks indicate absent amino acids. The underlined amino acids represent the signal peptide. The amino acid sequence data for the Par j 1.0101 are from Costa et al. [3].

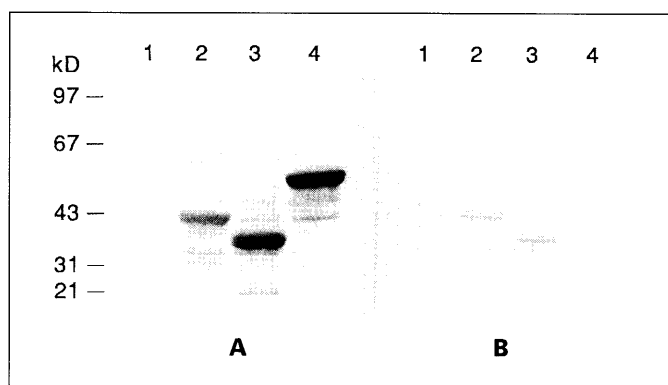


Fig. 3. Immunoblot analysis of recombinant proteins. Each lane was loaded with 10 µg of *E. coli* lysate. The blots were probed with pooled allergic human sera (**A**), or with pooled nonallergic human sera (**B**). Lanes: 1 = Non-induced recombinant lysate; 2 = lysate of induced pGEXP9 clone; 3 = lysate of induced pGEXP1 clone; 4 = lysate of induced pMALP5 clone.

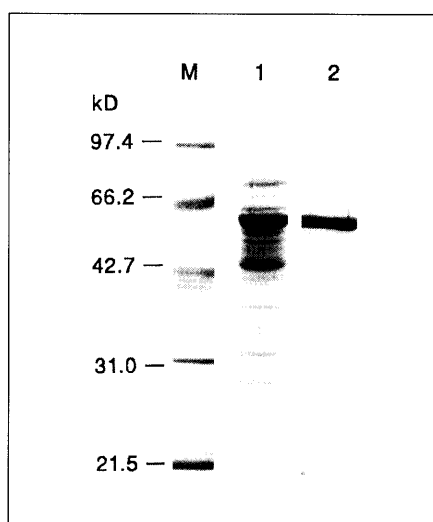


Fig. 4. Purification of the recombinant protein Par j 1.0101. The recombinant protein was purified as described in the Materials and Methods section, separated by 10% PAGE-SDS and stained with Coomassie brilliant blue. M = Protein markers (Biorad); 1 = cellular lysate; 2 = isolated recombinant protein Par j 1.0101.

was also a good immunogen because when the purified pMALP5 protein was injected in rabbits it was capable of inducing polyclonal antibodies as demonstrated by the CIE and CRIE results reported in figure 6, where the single arc obtained was capable of binding IgE specific for Pj allergens. The fusion of the allergen to the MBP protein does not appear to interfere with its capacity of interacting with the

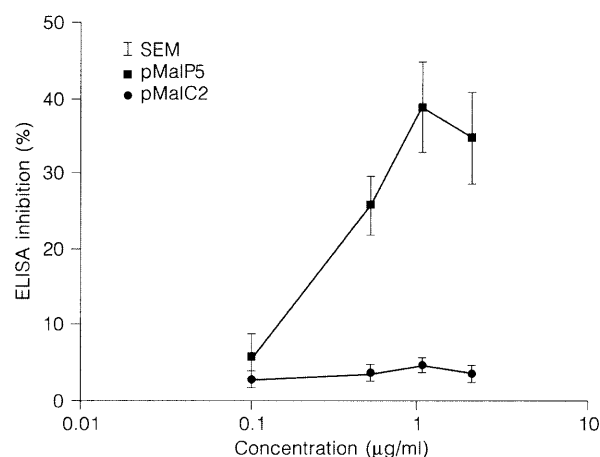


Fig. 5. ELISA inhibition assay. The curve represents the inhibition of the binding of IgE specific to the allergens present in the Pj pollen crude extract coated (0.1 µg/ml) to the microtiter wells. The inhibition was performed by using increasing amounts of pMalP5 *E. coli* lysate, while the pMalC2 *E. coli* lysate was used as negative control.

Table 1. Histamine released from stimulated basophils

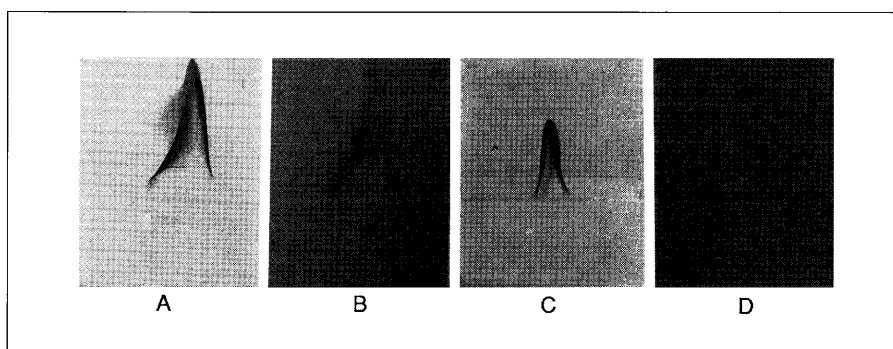
Subject	Crude extract	pMalP5 protein	Anti-IgE
R.P.	14	30	18
G.C.	27	19	23
G.D.	42	49	33
V.M.	30	29	38
I.D.	26	9	35
P.M.	55	56	32
D.G.*	0	0	46

The concentration of the stimuli used was 1 µg/ml for the Pj crude extract and the purified recombinant allergen pMalP5 and 10 µg/ml for the anti-human IgE. D.G. was a nonallergic subject used as a control. The values of HR are expressed as percentage and calculated as:

$$\frac{\text{stimulus} - \text{spontaneous release}}{\text{total release} - \text{spontaneous release}} \times 100.$$

Pj-specific IgE as suggested by the positive CRIE reported in figure 6B. Moreover, the bound IgE were specific for the Par j 1.0101 as suggested by the negative CRIE obtained when the MBP was used alone (fig. 6D).

Fig. 6. CIE and CRIE analysis of the Par j 1.0101. Examples of CIE (**A, C**) and CRIE autoradiography (**B, D**) of 10 µg of Par j 1.0101 fused to the MBP protein (**A, B**) and 10 µg of MBP alone (**C, D**). 50 µl of rabbit anti Par j 1.0101–MBP were loaded in the upper gel. **D** No cross-reactivity between MBP and IgE from allergic patients was found.



Discussion

The cloning and sequencing of allergenic molecules is a crucial strategy to study one of the fundamental components of the allergic reaction. The microheterogeneity of allergens has been reported for most of the allergenic sources so far analyzed [6, 10, 14, 24]. In the present paper, we report the isolation and characterization of two cDNA clones coding for two isoforms of the major Pj pollen allergen Par j 1.0101. The screening of a cDNA expression library gave ten positive IgE-binding plaques, and after a further purification step only two clones (P1 and P9) showed independent full length inserts. The analysis of the deduced amino acid sequence of the two cDNAs, Par j 1.0102 and Par j 1.0201, showed at the NH₂-terminal region a sequence of 37 and 36 amino acids, respectively, that represents a putative signal peptide characteristic of glycosylated eukaryotic proteins. Glycosylation of the Pj allergens has been already demonstrated in the past [25]. In particular, sequence comparison analysis between the Par j 1.0101 and the Par j 1.0102 proteins showed that the putative mature processed protein codified by the P9 clone presents 99% homology at nucleotide level and 96% at amino acid level. In addition, the 3' untranslated region of the P9 clone is identical to the corresponding region of the P5 clone (fig. 1A, B) suggesting that the P9 clone represents a full-length isoform of the already published Par j 1.0101.

The homology between the P5 and P1 clones was 91% at nucleotide level and 89% at amino acid level and then, according to the allergen nomenclature suggested [23], the Par j 1.0201 can be considered as a variant form of the major Par j 1.0101 allergen. Interestingly, the 5' and 3' untranslated regions of the P1 clone did not show any homology with the corresponding regions of the P5 clone, suggesting that the two isoforms are probably transcribed by two different genes. Computer search analysis suggested that the Pj re-

combinant allergens described in this paper belong to a family of plant proteins named as nonspecific lipid transfer protein [26, 27]. These proteins belong to a small multigene family capable of transferring lipid molecules through the membrane. They present common characteristics since all of them show a low molecular weight, a relevant homology at the amino acid level and a putative signal peptide at the NH₂-terminal region [28].

To study the immunological relevance of the recombinant Par j 1.0101, Par j 1.0102 and Par j 1.0201 proteins, we decided to express them in the pMalC2 and PGEX *E. coli* vectors. A Western blot analysis was performed using a pool of sera of Pj-allergic patients showing that the recombinant proteins were capable of binding human IgE (fig. 3). In sera from subjects not allergic to the Pj pollen used as a control, no reliable IgE binding was detected. Further, considering the very amino acid homology among the tree clones and the property that they reacted with the IgE from Pj-allergic patients and with a monoclonal antibody specific for the Par j 1.0101 (data not shown), we decided to analyze in more detail the allergenic activity of only one clone, P5, since it codifies for the putative mature major allergen. The rPar j 1.0101 protein was capable of inducing HR from basophils of 6 patients allergic to the Pj pollen. The amount of histamine released with the recombinant Par j 1.0101 was comparable to that obtained with the native Pj crude extract, suggesting a quite strong allergenic activity (table 1). The HR observed was due to an IgE-specific interaction with the recombinant allergen and not to a toxic effect since when basophils from subjects nonallergic to Pj were used, no histamine was detected. The interaction was really due to the recombinant Par j 1.0101 allergen since the allergen used for HR was purified at homogeneity (fig. 4).

An additional important property of the recombinant Par j 1.0101 allergen is its capability of inhibiting 40% of the IgE specific in an ELISA assay (fig. 5). The immunogenicity of

the recombinant Par j 1.0101 allergen was also well established after its capability of inducing the production of specific rabbit polyclonal antibodies. It was not restricted by the fusion to the MBP protein and the IgE Pj specific bound to the recombinant allergen and not to the fused protein, as suggested by the CRIE analysis.

In conclusion, in this paper we describe the isolation of two highly conserved isoforms of the major Pj allergen and its immunological characterization. The data suggest that

the recombinant Par j 1.0101 allergen may represent a reliable candidate for a diagnostic and a therapeutic approach to the Pj-allergic reaction.

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